

d) expressing the modified nucleic acid sequence in a host cell to produce the variant pullulanase.

2. (Amended) The method of claim 1, wherein the altered property is pH dependent activity, thermostability, substrate cleavage pattern, specific activity of cleavage, substrate specificity or substrate binding.

3. (Amended) The method of claim 2, wherein the altered property is a higher isoamylase activity as defined by an increase of at least 5% in the number of reducing ends formed in an "assay for isoamylase-like activity" using 50 mM sodium acetate, a pH of 4.5, 5.0 or 5.5, a temperature of 60°C and when incubated with a 10% w/v rabbit liver glycogen solution for a period of 10 min.

4. (Amended) The method of claim 1, wherein the altered property is an improved thermostability as defined by differential scanning calorimetry (DSC).

5. (Amended) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased half-life ( $T_{1/2}$ ) of at least about 5% in a " $T_{1/2}$  assay for liquefaction", using a pH of 5.0 and a temperature of 95°C.

6. (Amended) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased residual enzyme activity of at least about 5% in an "assay for residual activity after liquefaction", using a pH of 5.0 and a temperature of 95°C.

7. (Amended) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased half-life ( $T_{1/2}$ ) of at least about 5% in a " $T_{1/2}$  assay for saccharification", using a pH of 4.5 and a temperature of 70°C.

8. (Amended) The method of claim 1, wherein the altered property is an improved thermostability as defined by an increased residual enzyme activity of at least about 5% in an "assay for residual activity after saccharification", using a pH of 4.5 and a temperature of 63°C.

9. (Amended) The method according to claim 8, wherein the "assay for activity for saccharification", is carried out at a pH of 4.5 and at a temperature of 70°C.

10. (Amended) A method for constructing a variant of a parent pullulanase, the method comprising:

- a) identifying an internal or external cavity or crevice in a three-dimensional structure of the parent pullulanase;
- b) substituting at least one amino acid residue in the neighborhood of the cavity or crevice with another amino acid residue which increases the hydrophobic interaction and/or fills out or reduces the size of the cavity or crevice;
- c) optionally repeating steps a) and b) recursively;
- d) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b);
- e) preparing the variant resulting from steps a) - d);
- f) testing the thermostability of said variant; and
- g) optionally repeating steps a) - f) recursively; and
- h) selecting a variant having increased thermostability as compared to the parent pullulanase.

15. (Amended) A method according to claim 10, wherein the increased thermostability is as defined in claim 4.

20. (Amended) A method according to claim 1, wherein the parent pullulanase has more than 40% homology with the amino acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5.

21. (Reiterated) A method according to claim 20, wherein the parent pullulanase has the amino acid sequences shown in SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5.

22. (Amended) A method for producing a pullulanase variant, the method comprising:

- a) constructing the variant by the method according to claim 10;
- b) transforming a microorganism with a DNA sequence encoding the variant;
- c) cultivating the transformed microorganism under conditions which are conducive for producing the variant; and
- d) optionally, recovering the variant from the resulting culture broth.